THE ANNALS

AND

MAGAZINE OF NATURAL HISTORY.

[FOURTH SERIES.]

No. 105. SEPTEMBER 1876.

XVIII.—*The Development of the Ova of* Chthonius *in the Body of the Mother, and the Formation of the Blastoderm.* By Anton Stecker *.

The investigation of the first stages of development of the ova of *Chthonius* † possesses a much greater interest than would be supposed from what has hitherto been published upon it; for, except the memoir on the developmental history of *Chelifer* published by Metschnikoff ‡ in the year 1871, I can cite no other work containing any details of the embryology of the Chernetidæ. But as Metschnikoff only had scanty materials, the first stages of development observed by him, so far as they relate to the formation of the deutoplasmatic nutritive vitellus and the blastoderm, are so briefly described, that his work (as, indeed, he himself remarks §) remains very imperfect in this respect. I was fortunate enough to be able to obtain a great number of egg-bearing females of *Chthonius*, and thus had the opportunity of thoroughly investigating the first phases of those ova which are still in the ovary and therefore had to be obtained from the body of the mother by preparation; but as I had at the same time brought up a number of other females in captivity, I was placed in the favourable position of being able to examine simultaneously and

* Translated by W. S. Dallas, F.L.S., from a separate impression of the memoir published in the 'Sitzungsberichte der königl. böhm. Gesellsch. der Wiss.' 1876, Heft 3.

† *Chthonius*, a genus of the order Chernetidæ, family Obisinæ; *Chelifer*, also a genus of Chernetidæ, of the family Cheliferinæ.

‡ Zeitschrift für wiss. Zool. Bd. xxi. (1871) pp. 513–526, pls. 38 & 39.

§ Metschnikoff, *l. c.* p. 514.

thoroughly the freshly deposited eggs attached to the ventral surface. These were placed in an inodorous oil, where they continued their development for a time; so that I was enabled to attain a satisfactory notion of the rapid process of development (the so-called segmentation) of the egg, and of the first formation of the blastoderm. The eggs and embryos hardened in absolute alcohol also furnished very good objects of observation.

In this preliminary communication I will only call the reader's attention to the most interesting points, without entering into details, reserving the detailed description of the entire process of development, with figures of the particular formative phases, for a later publication.

In general we must distinguish three principal phases in the development of *Chthonius*. The first of these embraces those egg-formations which take place in the body of the mother; the second includes the metamorphosis of the fresh-laid eggs up to the complete development of the blastoderm—that is, to the first change of skin; and the third and last phase is presented by those changes which occur in the newly hatched larvæ (analogous to the *Nauplius* stage) on the ventral surface of the mother.

In this preliminary communication we shall take into consideration only the first and second phases, as these have not been described by Metschnikoff with the same accuracy as the third phase, the larval stage of *Chelifer* *.

The ovary forms an unpaired gland, which has already been correctly described and figured in the Chernetidæ by Menge †; the individual ova, which become larger and more and more fitted for deposition the nearer they are to the paired oviduct, give to the ovarian gland a racemose form. As has already been correctly remarked by Menge ‡, the female genital organs open by two orifices at the second abdominal segment; the apertures are placed very near together. A depression situated in front of them serves to provide the deposited eggs with a sticky mass, secreted by a gland which opens here.

The youngest ovicells are found imbedded in the interior of the ovary. During the further development of the ovicell (consisting of protoplasm, Purkinje's vesicle, and germinal spot), which takes place in the same way that has already been described by Metschnikoff in the eggs of the scorpion §, the wall

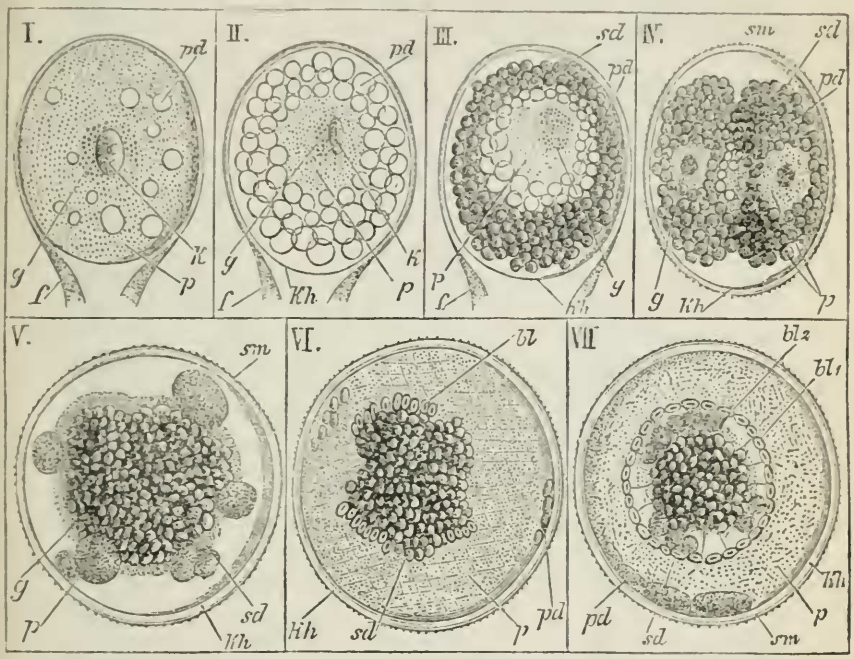
* Metschnikoff, *l. c.* pp. 518–522.

† A. Menge, "Ueber die Scheerenspinnen, Chernetidæ," Neueste Schriften der naturf. Gesellsch. zu Danzig, v. (1855), 2, p. 17, pl. 2. fig. 10.

‡ Menge, *l. c.* p. 17.

§ Metschnikoff, "Embryologie des Scorpions," Zeitschrift für wiss. Zool. Bd. xxi. (1871), pp. 204–232, pls. xiv.–xvii.

of the ovary is pushed outwards in the form of a round eminence, giving rise to the peculiar racemose form of the ovary. As the development of the ova advances, the wall of the ovary is pushed still more outwards, so that then each ovum is enclosed in a perfectly homogeneous follicle * (therefore without any epithelial layer), the basal section of which appears in the form of a short pedicle lined with nucleated cells arranged in a spindle-like form.



Embryonal development in *Chthonius*, represented in seven successive stages: bl_1, blastoderm (first layer); bl_2, mesodermic cells (?); *f*, follicle; *g*, coarser granules of the protoplasm; *k*, Purkinje's vesicle; *kh*, vitelline membrane; *p*, protoplasm; *pd*, primary deutoplasm-spheres; *sm*, secondary membrane; *sd*, secondary deutoplasm-spheres (nutritive vitellus).

The ovum is now developed chiefly by a rapid increase of volume of the protoplasm, in which we must distinguish two kinds of granules, coarser and finer. The coarser granules

* Von Wittich, 'Observationes quædam de Aranearum ex ovo evolutione,' Diss. inaug. Halis Saxoniæ, 1845; and *id.* "Die Entstehung des Arachnideneies im Eierstock, die ersten Vorgänge in demselben nach seinem Verlassen des Mutterkörpers," Müller's Arch. für Anat. und Physiol. 1849, pp. 112–150, pl. iii. (see p. 116).

collect by degrees around the germinal vesicle (fig. I., *g*); whilst the finer ones are uniformly distributed in the whole mass of protoplasm, the true formative vitellus. The ovum is enveloped by a simple structureless membrane, the vitelline membrane; an external secondary membrane only makes its appearance subsequently. Even before this stage the ovum, *i.e.* the protoplasm-mass of the ovum, becomes occupied by large, clear globules of albuminous appearance (figs. I.–IV., *pd*), which appear first at the pedicular pole, but then rapidly accumulate round the germinal vesicle, which is situated in the middle of the ovicell. Whether these spheres, which we may call *primary* deutoplasm-spheres, originate from the syncytium (Häckel) of the ovary*, is more than I can say, as, notwithstanding all my endeavours, I could not trace their formation; the whole process takes place so rapidly, that the whole ovum seems to be at once completely filled with these deutoplasm-spheres. At the same time the limpid germinal vesicle acquires a fusiform shape, until at last it is completely surrounded by the primary deutoplasm-drops. A section (fig. II.) through the ovum when in this stage will convince us that around the vesicle of Purkinje (which, it may be remarked in passing, gradually diminishes and finally disappears altogether) a portion of protoplasm filled with numerous fine granules has accumulated. In the middle, almost in the place of the vanishing germinal vesicle, a round brown spot now becomes visible, composed of the coarser granules of the protoplasm; this explains the concentration of the coarser granules of the protoplasm from their first appearance in the ovicell.

In this stage of development a new, peculiar process commences. A portion of the primary albuminoid deutoplasm-drops gradually coalesce and become converted into a number of strongly refractive deutoplasm-drops of fatty appearance (we may characterize these formations as *secondary* deutoplasm-spheres), which are, indeed, smaller than the primary deutoplasm-spheres, but soon so multiply that, even in a very short time and whilst the volume of the ovum increases, it appears completely crammed with the secondary deutoplasm-drops (fig. III., *sd*). A section made through the middle of the ovum (fig. III.) would now show its composition to be as follows:—In the middle of the ovum, instead of the Purkinjean vesicle, which has entirely disappeared, there is the round brown nucleus consisting of coarser protoplasm granules, which, as we shall see hereafter, play a very important part

* Dr. Bertkau, "Ueber den Generationsapparat der Araneiden," Archiv für Naturg. Bd. xli. (1875) pp. 235–262, pl. vii. (see p. 245).

in the formation of the blastoderm; this is surrounded by a tolerably voluminous, finely granular layer of protoplasm. The protoplasm layer is followed by an inconsiderable layer of primary deutoplasm-drops, which again is surrounded by a voluminous layer of the secondary deutoplasm-drops, "the true nutritive vitellus." This stage is the last that I could meet with in the interior of the female; in a few specimens I further detected an invagination of the nutritive vitellus at the two poles of the ovum; but it is certain that this stage represents an ovum ready for deposition.

When the ova are ready for deposition they pass through the pedicle (the cells of which, as in the true spiders *, are not very perceptible, perhaps also in consequence of the secreted deutoplasm-spheres?) into the internal cavity of the ovary. That in the Chernetidæ, as in the Araneidæ, the ova do not really fall off from the pedicles, perhaps into the body-cavity (as might be supposed with regard to the true spiders from Leydig's †, and with regard to the Chernetidæ from Metschnikoff's ‡ figures), but pass through the pedicle into the oviduct, has already been observed by Menge §, whose investigations I can only confirm; for an ovary dissected out of a female which had already deposited her eggs was abundantly beset with empty follicles. The oviducts opening in the second abdominal segment may also serve to convince us of this. The process of oviposition was observed by Leuckart ‖ in the Pentastomes; and as it is in general precisely similar to that of *Chthonius*, we shall not occupy any more time with its description. Metschnikoff ¶ also observed this process in the scorpions; and in these also it resembles that of the Pentastomes.

No doubt owing to his scanty materials, Metschnikoff did not observe the very first metamorphosis preceding the development of the true nutritive vitellus; and hence, of course, the true origin of the vitelline spheres (which, as he remarks **, are "so characteristic of *Chelifer*") escaped him. Otherwise it is impossible that such great differences should occur in the development of two genera of one and the same order.

I must further remark that Metschnikoff might well state that

* Bertkau, *l. c.* p. 246.

† Leydig, 'Lehrbuch der Histologie,' p. 550, fig. 271.

‡ Metschnikoff, "Entwicklung des *Chelifer*," *l. c.* pl. xxxviii. figs. 1 & 2.

§ Menge, *l. c.* p. 17, pl. ii. fig. 10.

‖ Leuckart, 'Bau und Entwickelungsgeschichte der Pentastomen,' Leipzig und Heidelberg, 1860, p. 84.

¶ Metschnikoff, "Embryologie des Scorpions," *l. c.* pp. 208, 209.

** Metschnikoff, "Entw. des *Chelifer*," *l. c.* p. 514.

the peculiar round bodies (spheres) which, as he says*, occur in the protoplasm of the spider's ovum, are wanting in *Chelifer* (as in the ovum of the true scorpions). But I believe that in the secondary deutoplasm-spheres we may see an analogue of the spheres observed by Claparède †, Zalensky ‡, Balbiani §, and others, but first correctly understood as deutoplasm-spheres by Ludwig ‖, and which afterwards become the peculiar flakes (*Schollen*).

The freshly deposited egg, attached to the ventral surface of the mother, measures from 0·095 to 0·12 millim., is of an elongate ovate form, and presents, besides the so-called vitelline membrane secreted by the ovicell while still in the ovary, a secondary layer, which was separated from the protoplasm layer of the ovarian tube ¶ during the deposition of the egg (at least this is the only possible explanation of it). Like the secondary external membrane of the eggs of *Philodromus* investigated by Ludwig **, this is divided into rounded areas, which give the entire membrane an elegant cellular appearance, and are produced by the fine granules of the protoplasm-mass being arranged in circles. They were observed and figured by Metschnikoff ††, who, however, says nothing about their origin.

The egg in this stage is now subjected to a new and very important process: the segmentation, which closely resembles that of *Chelifer*, commences; and here also the nutritive vitellus undergoes a total segmentation (*amphigastrula*, Häckel ‡‡).

* "Entw. des *Chelifer*," *l. c.* p. 575; "Embr. des Scorpions," *l. c.* p. 208.

† E. Claparède, "Recherches sur l'évolution des Araignées," Utrecht 1862, in Natuurk. Verh. Utrechtsch Genootschap van Kunsten en Wetensch. Deel i.

‡ Zapiski Kieffskaro Obshtchestva Estestvoispitatelei, tom. ii. (1871) pp. 1–72, with 3 plates.

§ Balbiani, "Mémoires sur le développement des Aranéides," Ann. des Sci. Nat. 5e sér. Zool. tome xviii. (1873) art i., with 15 plates.

‖ Hubert Ludwig, "Ueber die Bildung des Blastoderms bei den Spinnen," Zeitschr. für wiss. Zool. Bd. xxvi. (1876) pp. 470–485, pls. xxix., xxx.

¶ The inner wall of the ovary in *Chthonius* is lined by a layer of homogeneous protoplasm-mass in which numerous nuclei are imbedded, but without the individualization of any special portions of protoplasm around the nuclei (*syncytium*, Häckel).

** Ludwig, *l. c.* p. 471, pl. xxx.

†† Metschnikoff, "Entw. des *Chelifer*," *l. c.* p. 516, pl. xxxviii.

‡‡ E. Häckel, "Die Gastrula und die Eifurchung der Thiere," Jenaische Zeitschr. für Naturw. Bd. x. (1876) pp. 61–167, pls. ii.–viii. (see pp. 67 and 83 *et seqq.*).

Häckel well remarks that the unequal segmentation occurs in by far the greater number of Arthropoda, but in most cases has not been accurately enough observed.

The segmentation takes place quite regularly and very rapidly; the nutritive vitellus breaks up into two, then into four, and finally into eight segments (segmentation-spheres). The division takes place by the formation of invaginations at the two poles of the egg, appearing first at the pedicular pole. It is true that I have found an ovum with invaginated poles even in the ovary; but the first indications of the process of segmentation, in by far the greater number of cases, are only to be met with on the ventral surface of the mother, so that the segmentation in *Chthonius* is a process occurring in deposited eggs. With the division of the nutritive vitellus, the brown nucleus, which is pushed a little to one side, also breaks up into two halves, one of which pertains to each segment; but with this at the same time a division of protoplasm is closely connected, and, indeed, the protoplasm divides into three portions; one part occupies the cavity between the two segmentation-spheres and remains there, surrounded by a layer of the primary deutoplasm-spheres, throughout the whole process of segmentation. Of the rest of the protoplasm, nearly equal portions collect round the nuclei of the two vitelline segments. We have then in *Chthonius* an internal cavity which is at the same time a reservoir of protoplasm, to be afterwards separated from this cavity in order to surround the nutritive vitellus (fig. IV.).

Now a further division of the two spheres of segmentation into four takes place, being effected by a transverse invagination of the two vitelline cells. Both in this and in the following stage, in which the vitellus is divided into eight segments, the nucleus, and consequently also the protoplasm, likewise divides into four and then into eight parts, so that in the last-mentioned stage eight segmentation-spheres, each with a nucleus which is surrounded by a layer of protoplasm, may be distinguished. The structures detected by Metschnikoff* in the eggs of *Chelifer* with four so-called spheres of segmentation, namely the round brown spots consisting of fine granules (representing the cell-nuclei according to Metschnikoff), are therefore to be regarded as equivalent to the nuclei composed of the coarser protoplasmatic granules.

When the vitellus has passed through the process of segmentation up to this point, a new and very important process commences—namely, the separation (*Ausscheidung*) of the protoplasm, which is, so to speak, a preparatory process to the formation of the blastoderm. After the vitelline membrane has removed considerably from the large vitelline cells situated in the centre of the egg, several protoplasm-spheres, which,

* Metschnikoff, "Entw. des *Chelifer*," *l. c.* p. 515, pl. xxviii. figs. 4–7.

when examined under a high power, appear to be filled with very fine granules, become perceptible in the egg. The protoplasm-spheres increase more and more, until at last they form a continuous voluminous layer around the vitelline spheres, which are greatly reduced in volume (fig. V.). At the same time we have an equally important process to mention, namely the gradual dissolution of the nuclei contained in the spheres of segmentation; under the microscope we can very well follow the breaking-up of the individual nuclei into a great number of granules.

With regard to the origin of the protoplasm-balls, which gradually increase in the egg, I agree with Metschnikoff: I believe that these have separated from the large spheres of segmentation; only I may remark that by this I understand not the protoplasm-mass occurring in the individual spheres, but the protoplasm collected in the reservoir, which has separated itself. An analogous formation, a separation of protoplasm, occurs also in many Gasteropoda, Ctenophora, Planariæ, &c.* It is possible that in these animals also the whole process takes place in the same way as in *Chthonius*—namely, that in them also a portion of protoplasm is preserved through the whole course of segmentation in a central cavity, and afterwards separated therefrom.

With the separation of the protoplasm the spheres of the primary deutoplasm confined in the central cavity also come into view, with their form indeed a little altered, but still quite recognizable in their origin as primary deutoplasm-spheres. These collect at the periphery of the egg, where they gradually constitute an albuminous-looking layer composed of a great number of small spherules (figs. VI. & VII., *pd*). This is the same layer which was indicated by Metschnikoff† "as perhaps a kind of embryonal envelope;" with regard to its origin Metschnikoff says nothing further. It seems to me improbable, however, that this layer represents an embryonal envelope, and, indeed, for the same reason which is given in passing by Metschnikoff. What function pertains to this structure, which is apparently constant in the Chernetidæ, is partially revealed

* See Ray Lankester, "Observations on the Development of the Pond Snail," Quart. Journ. Micr. Sci. vol. xiv. 1874; Carl Rabl, "Die Ontogenie der Süsswasser-Pulmonaten," Jen. Zeitschr. für Naturw. Bd. ix. (1875) pl. vii.; W. Flemming, "Studien in der Entwicklungsgeschichte der Najaden," Sitzungsb. Wien. Akad. Bd. lxxi. (1875); A. Agassiz, 'Embryology of the Ctenophora,' Cambridge, Mass., 1874; and A. Kowalevsky, "Embryologische Studien an Würmern und Arthropoden," Mém. Acad. St. Pétersb. tom. xvi. (1871).

† Metschnikoff, "Entw. des *Chelifer*," *l. c.* p. 216, pl. xxxviii. figs. 7 & 8, *al.*

when we carefully consider the subsequent embryonal stages of *Chthonius*. I believe that I am not wrong in thinking that it is the same substance which in a later stage of the embryo, in which the first rudiments of the extremities occur, occupies the internal cavity of the embryo. In fact the formation of the albuminoid mass in the interior of the embryo can only be explained by a penetration of the albuminous-looking layer into it; the rapid diminution of the layer in the stage just mentioned is in accordance with this.

As I have already mentioned, the nuclei of the spheres of segmentation break up partially into a number of granules. In this, however, consists the first step towards the formation of the true blastoderm; for the granules become surrounded by corresponding portions of protoplasm—a process which represents the first formation of the subsequent blastodermic cells in the interior of the nutritive vitellus. Just as in the eggs of *Philodromus* (Ludwig *), the protoplasm-spherules must here also work out through the deutoplasm of the nutritive vitellus to its outer surface, which, in fact, really happens; and the nutritive vitellus (analogously to the portions of deutoplasm converted into flakes (*Schollen*), Ludwig) is already surrounded by a continuous layer of protoplasm-balls, each of which is provided with a distinct nucleus (figs. VI., *bl*, & VII., bl_1). The protoplasm-spherules thus produced arrange themselves on the surface, and by mutual approximation and limitation form the blastodermic vesicle; the protoplasm-balls become more and more individualized, and finally form distinctly marked blastodermic cells (fig. VII.). The blastoderm is then further developed: the cells which have hitherto been separated arrange themselves, so to speak, after the fashion of pavement-epithelium, and gradually separate from the nutritive vitellus, whilst at the same time the interspace thus produced begins to fill with a new layer of blastodermic cells. These latter cells (mesodermic cells?) are larger than those already mentioned, rounded, and generally full of granules, and accumulate (as Metschnikoff has already remarked) upon the same part of the embryo on which the provisional appendage, described as the "lip-muscle" by Metschnikoff †, afterwards occurs (fig. VII., bl_2).

Thus I have reached the end of the description of my results relating to the developmental history of the ovum in the ovary and the formation of the blastoderm of *Chthonius*. What has been said may be briefly summed up as follows:—

The protoplasm gradually becomes filled with primary deuto-

* Ludwig, *l. c.* pp. 477 *et seqq.*

† Metschnikoff, "Entw. des *Chelifer*," *l. c.* p. 517. pl. xxxviii. fig. 9 *c*.

plasm-spheres; these collect round the germinal vesicle, which is situated in the centre and surrounded by a layer of protoplasm. The germinal vesicle disappears. The primary deutoplasm-spheres become secondary ones—the true nutritive vitellus, which contains in its interior first a brown nucleus consisting of granules separated from the protoplasm, then a layer of protoplasm, and lastly a layer of untransformed primary deutoplasm-spheres. Now the segmentation takes place, and is total: the nutritive vitellus divides into two, four, and finally eight large vitelline cells; at the same time the nucleus and the protoplasm also divide. An internal cavity also is formed, in which a portion of the protoplasm is preserved; after the completion of the segmentation this separates outwards and envelops the nutritive vitellus. With the protoplasm the primary deutoplasm-spheres confined in the same cavity also come into view; and these then form an albuminous-looking layer at the periphery of the egg. Next the nuclei of the individual vitelline spheres break up partially into a number of granules, and work, with the protoplasm surrounding them, out of the vitelline cells, which are constantly more and more reduced, arrange themselves superficially, become individualized as independent cells, and thus form the blastodermic vesicle.

If we now compare these details, especially with respect to the formation of the blastoderm, with the results of Ludwig's investigations of the formation of the blastoderm in the egg of *Philodromus**, we at once see the great analogy that exists between the two processes; for Ludwig's deutoplasm-spheres, which unite into columns and afterwards develop into the peculiar flakes (*Schollen*), correspond to the secondary deutoplasm-spheres of *Chthonius*. The nuclei originating in the central substance of the rosettes (the protoplasm of *Chthonius*),

* We may be permitted here to recapitulate briefly Ludwig's extremely interesting results (*l. c.* p. 479):—"The germinal vesicle disappears; the deutoplasm-spheres unite to form columns, which group themselves radiately around a central protoplasmatic substance and are held together by it; this rosette divides binarily into several rosettes of division; nuclei originate in the central substance of the rosettes; the nuclei with the protoplasm surrounding them work out of the rosettes, which during their division are constantly pressed more and more towards the periphery, arrange themselves superficially, and form by mutual approximation and limitation the blastodermic vesicle; the portions of deutoplasm which have become flakes (*Schollen*) sink back into the interior of the egg."

are not they a distinct analogue of the nuclei of the vitelline cells separated from the protoplasm in *Chthonius*? As in *Philodromus*, so also in *Chthonius*, and we may fairly assume in *Chelifer* likewise, a portion of the broken-up granules with the portions of protoplasm surrounding them work out of the vitelline spheres to their surface, whilst the other part, with the dentoplasm, becomes the entoderm.

In *Chthonius*, as in *Philodromus*, a total and, indeed, "unequal" segmentation takes place, such as we also meet with elsewhere. For if we consider the amphigastrula of *Purpura* (according to Selenka *), or the amphigastrula of *Petromyzon* (according to Schultze†) and of *Bombinator* (according to Götte ‡), or, lastly, the amphigastrula of *Fabricia* or *Trochus* (according to Häckel §), and compare them with the amphigastrula of *Chthonius* (see fig. VII.) or *Chelifer* (according to Metschnikoff ‖), the close resemblance of all these structures is at once perceptible.

Thus we find an agreement between the amphigastrula of Chthonius, *or rather of the Chernetidæ, and the corresponding embryonal structures not only of the Vermes and Arthropoda, but also of the Mollusca and Vertebrata.*

In the amphigastrula of *Chthonius*, indeed, I have been unable to observe the primitive mouth; possibly it is stopped by a vitelline plug, as is the case in the amphigastrula of *Bombinator* according to Götte.

The eggs of the Chernetidæ therefore furnish a new and good contribution to the formation of the amphigastrula; and we must once more repeat Häckel's words, "that the unequal segmentation is tolerably widely diffused among the Arthropoda, but in most cases has not yet been accurately observed." Moreover, by these results the investigations of Van Beneden and Bessels ¶ are again confirmed; according to them, in the different segmentations of the egg of the Arthropoda an extended series of transition forms occurs leading from one mode of segmentation to the other. *The segmentation of* Chthonius, *although "unequal," yet in many respects resembles the "superficial."*

* Selenka, "Keimblätter bei *Purpura*," Niederl. Arch. für Zool. 1871, Heft 2, pl. xvii.

† M. Schultze, 'Entwicklungsgeschichte von *Petromyzon*,' Haarlem, 1856, pl. iv. figs. 5 & 7.

‡ Götte, 'Keimesgeschichte der Unke,' Leipzig, 1875, pl. ii. fig. 33.

§ Häckel, *l. c.* pl. vii. figs. 100 & 110.

‖ Metschnikoff, "Entw. des *Chelifer*," *l. c.* pl. xxxviii. fig. 9.

¶ E. van Beneden et Emil Bessels, "Sur la formation du Blastoderme chez les Crustacés," Bull. et Mém. de l'Acad. Belg. 1868, 1869.